METHOD OF MATCHING RETENTION TIMES AMONG MULTIPLE CHROMATOGRAPHIC SYSTEM

Technical Field

The technical field generally relates to chromatographic systems and in particular to methods for matching retention times among multiple chromatographic systems.

Background

Chromatography (gas, liquid, electro-driven) is a powerful analytical tool that can separate, identify, and quantify multiple analytes in a single analysis. The principal components of a typical chromatographic system include (1) an inlet that provides the interface to transfer a sample mixture into the chromatographic (separation) column; (2) a separation column that separates the sample mixture into its individual components as these components are swept through the column by a mobile phase; (3) a mobile phase to provide a driving force to move solutes from one end of the column to the other, the separation being based on a combination of differential interactions between the components of the sample mixture, an immobilized liquid or solid material within the column (stationary phase) and mobile phase, and (4) a detector that detects and measures components as they exit the separation column at different times. The exit time of a component is defined as the "retention time (RT)" for that component. Some chromatographic methods are capable of separating more than two hundred components in a single analysis. However, for chromatographic methods involving large numbers of components, a significant amount of work is required to determine the RT of each individual component during chromatographic method development. Also significant is the amount of work needed to correlate data generated on multiple instruments performing the same analysis, even for a small number of components.

The problem of replication arises after the chromatographic method development is completed. There are several parameters that affect RT. These include column parameters (e.g., length, stationary phase, particle size, and inside diameter) as well as operating parameters for the chromatograph (e.g., mobile phase type and flow rate, column temperature, ramp rates, column outlet pressure, and stationary phase thickness). Whenever a chromatographic method is used subsequent to its development, it is virtually impossible to replicate all the parameters precisely enough to obtain exactly the same retention times as those observed initially. The cumulative effects of these small but finite differences in parameters usually lead to significant differences in RTs. As an example, when two "identical" gas chromatography (GC) systems were set up to run the

1   same chromatographic method on the same pesticide samples, the RTs for specific solutes

2   were different by 0.5 minutes for peaks eluting at 20 minutes.

3   Without exact replication, measured RTs do not match the RTs specified in the

4   original chromatographic method or the computerized method files (including calibration

5   and event tables) and can lead to misidentified peaks with grave consequences in

6   applications such as forensic, clinical or environmental analysis. The need therefore exists

7   for means to remove or easily compensate for these RT differences.

8   *Prior solutions and their disadvantages*

9   There exist two general ways improving the match of RTs over time and between

10   one system and another: instrumental and calculational. Instrumental approaches seek to

11   reduce differences in RTs by adjusting one or more instrumental parameters such as flow

12   rate and temperature program rate. As a consequence of instrumental approaches, the

13   actual retention times that are generated during analysis more closely match reference

14   RTs.

15   In calculational approaches, the actual RT data are modified after the RT data are

16   acquired. The most obvious and widely used calculational method for dealing with RT

17   mismatch in a situation subsequent to that of the reference analysis is to re-run a

18   mixture(s) containing all of the possible compounds to be analyzed to determine

19   individual RTs in the new situation. This is a reasonable task for simple chromatographic

20   methods with a small number of well-separated analytes. However, this process becomes

21   much more difficult and time consuming as the number of analytes increases or when

22   using different chromatograph configurations. In addition, this approach does not address

23   the differences between the RTs obtained in a target chromatographic system and those in

24   reference libraries and databases, nor does the approach help in visual or mathematical

25   comparison of chromatographic data obtained on other instruments.

26   A popular "relative retention" calculational approach utilizes retention indices or

27   Kovats indices that circumvent problems in getting the same retention time from

28   instrument-to-instrument, column-to-column. This type of procedure converts the actual

29   retention times of detected peaks into a number that is normalized to (usually) multiple

30   reference compounds. The Kovats and other relative retention procedures are especially

31   useful for comparing retention times to databases and libraries for identification of

32   individual components. However, these procedures do not help in visual or mathematical

33   comparison of chromatographic data obtained on other instruments, because the

34   procedures adjust the retention times from the integration report and do not effect the

1. plotted chromatographic data or the integrated (slice) data. In addition, most retention
2. index calculations do not use a smoothed correction function, so the resulting indices rely
3. on the accuracy and reproducibility of retention times of reference peaks that bracket the
4. compounds of interest, and are therefore inherently less precise than when using a
5. smoothed correction function.
6.   Lantos et al. describe the application of a polynomial regression to facilitate
7. comparison of retention data from two different GC pesticide databases *(Lantos J. et al.*
8. *"Validation of gas chromatographic databases for qualitative identification of active*
9. *ingredients of pesticide residues" Principles and Practices of Method Validation*
10. *256:128-137, 2000).* Although Lantos et al. had some success at correlating the data,
11. this type of approach is fundamentally flawed. Specifically, the selected data used by the
12. authors came from methods with significant method differences. Changes in stationary
13. phase chemistry, temperatures and flows (outside the rules of method translation) that
14. form the basis of the Lantos approach lead to changes in relative as well as absolute
15. retention times of solutes. General mathematical approaches cannot correct for these
16. changes. Note that in the Lantos reference, the corrected retention times of almost all (18
17. out of 23) of the peaks selected for listing deviated by more than 1%, with three
18. exceeding 10%. The database searching time windows required for RT differences as
19. high as in Lanto's reference (windows > 1 min) would generate a prohibitively high
20. number of hits, rendering the approach unusable. In addition, there is no accommodation
21. for correcting peak response, scaling methods, or changing x-axis or y-axis units in
22. Lanto's method.
23.   An instrumental approach to matching GC retention times is described in U.S.
24. Patent No. 5,958,246 to Tipler et al. The Tipler technique somewhat improves the match
25. in RTs between systems, but the technique is a very involved, time-consuming procedure
26. and has proven to be limited in practical application.
27.   A more recent and advantageous instrumental approach to solving these problems
28. in GC is that of "retention time locking" (RTL). This technique, described in U.S. Patent
29. No. 5,987,959 to Klee et al., which is incorporated herein by reference, addresses the
30. problem of matching RTs on multiple systems.
31.   The Klee technique, referred to as the RTL I method hereafter, provides a method
32. for automated matching of retention times obtained using a known chromatographic
33. method having a defined set of column parameters and operating parameters to the
34. retention times obtained using a new chromatographic method having a new set of

1 column parameters, wherein the retention times of components separated in accordance

2 with the new chromatographic method are matched to the retention times set forth in the

3 known chromatographic method. A procedure is described to adjust head pressure to

4 compensate for differences in a new versus the original column, carrier gas, and column

5 outlet pressure of the known chromatographic method.

6   The use of the RTL I method to enhance identification of unknowns with the use

7 of RT databases is described in U.S. Patent No. 5,827,946 to Klee et al., which is

8 incorporated herein by reference.

9   The RTL I method makes significant improvements in the degree to which

10 retention times match between multiple systems and over time. All of the nine tasks

11 listed above and more are improved with the use of the RTL I method. There are,

12 however, shortcomings to the RTL I method. These shortcomings include:

13 1.   While the RT of the locking peak is often very well matched (typically to within

14   0.005 min), the resolution of pressure adjustment (0.01 psi) is often insufficient to

15   produce a match for the locking peak of better than 0.015 min for columns with inlet

16   pressures below 5 psi. For columns with higher inlet pressures (greater than 20 psi),

17   the match can usually be made to within 0.002 min.

18 2.   Even if the RT of the locking peak is precisely matched, the peaks located at RTs

19   significantly removed from the locking peak can still have significant RT differences

20   from other columns, instruments, libraries, or databases. These differences can be

21   large enough to cause misidentification of compounds and all of the other problems

22   associated with RT differences.

23 3.   In cases where method translation (described in U.S. Patent No. 5,405,432 to

24   Snyder et al.) is attempted using a column with a different phase ratio than the

25   original one, the current forms of RTL cannot match RTs with as high quality. They

26   deviate by amounts that are not easily predicted or compensated for experimentally.

27 4.   Some analysts use methods with two columns of different types connected to a

28 single injection port but to separate detectors. This approach allows for dual column

29 identification. Only one of these columns can be locked using RTL, however, since there

30 is only one pressure that can be set.

31 **Summary**

32   A method for correlating local chromatographic data to reference

33 chromatographic data, which for convenience will be referred to as the RTL II method, is

34 disclosed. Once a particular chromatographic method (the reference chromatographic

1   method) is developed and validated to satisfaction on a particular chromatographic

2   system (the reference system), a calibration mix of a plurality of calibration compounds

3   with RTs covering the complete RT range of the reference chromatographic method is

4   run. The calibration mix may also provide reference response factors for standard

5   compounds of interest. This process is referred to as the reference calibration, and

6   generates reference calibration data. When a user wants to implement the reference

7   chromatographic method on another chromatographic system (the local system), the user

8   sets up the reference chromatographic method in the local system and preferably, but not

9   necessarily, lock the reference chromatographic method using an instrumental correction

10 technique like the RTL I method. The user then runs the same calibration mix in the local

11 system to create local calibration data. This process is referred to as local calibration.

12 RTL II software uses a mathematical relationship between reference and local

13 calibrations to construct a "local system correction function," which is saved for

14 subsequent use to correct local chromatographic data. In the RTL II process, the peak

15 areas for locally generated chromatographic data are preserved in the corrected local

16 chromatographic data, even though the RTs have been adjusted by the RTL II process to

17 match the reference chromatographic data.

18   In an embodiment, the RTL II method includes transformation of the time axis of

19 the corrected local chromatographic data into another unit while maintaining the original

20 peak area for accurate quantitation. The transformed axis can possess equally spaced data

21 points (or any other function we choose), even if the relationship between those units and

22 RT is not linear. Examples include time axis transformation to: (1) retention index, which

23 is used extensively in the flavor industry; (2) boiling point, which is used in simulated

24 distillation analysis in the petroleum industry; (3) carbon number, which is of interest to

25 the petroleum industry; (4) molecular weight, which is usually of more of interest in

26 liquid chromatography, and (5) molecular size, which is usually of interest in capillary gel

27 electrophoresis.

28   In another embodiment, the y-axis of the local chromatographic data is

29 transformed during the transformation procedure. Examples include (1) simple scaling of

30 chromatographic response to remove differences in gain and offset between detectors on

31 different systems; and (2) use of a series of, for example, normal alkanes to correct for

32 systematic variations in response caused by phenomena like molecular weight

33 discrimination in the inlet.

1   In yet another embodiment, a reference library or database of RTs is transformed

2   so that the reference library or database is now adjusted to more directly correlate to a

3   characteristic of primary interest. Conversely, local chromatographic results can be

4   transformed to better correlate to the reference library or database of chromatographic

5   results.

6   In yet another embodiment, the construction of the local system correction

7   function and/or the application of this function to the local chromatographic data are

8   performed at a remote site through a network, such as a local network or the Internet.

9   In summary, the RTL II significantly improves the degree of RT matching and

10   response factor matching beyond that obtained normally or even after using the RTL I (or

11   other instrumental) method. The RTL II method can be used for improving RT matching

12   in all of the situations where the RTL I method can be used, as well as in situations where

13   the RTL I method is not applicable. A number of operations, such as searching,

14   matching, visual comparison, mathematical manipulation, and pattern recognition, will all

15   benefit from the RTL II method.

16   **Brief Description of the Drawings**

17   The detailed description will refer to the following drawings, in which like

18   numerals refer to like elements, and in which:

19   **FIG. 1a** is a flowchart showing the major steps in the RTL II method.

20   **FIG. 1b** depicts an local chromatographic apparatus 150 containing the RTL II

21   software.

22   **FIG. 2** is a flowchart showing the generation of a local system correction

23   function.

24   **FIG. 3** is a flowchart showing the application of the local system correction

25   function to local chromatographic data.

26   **FIG. 4** is a flowchart showing the use of the RTL II method with method

27   translation technique.

28   **FIG. 5** is a flowchart showing the use of the RTL II method with unit

29   transformation of x-axis.

30   **FIG. 6** is a flowchart showing the RTL II transformation of chromatograms

31   without the use of reference calibration mixtures.

32   **FIG. 7** is a flowchart showing the incorporation of response transformation in the

33   RTL II method.

1   FIG. 8 is a flowchart showing the use of the RTL II method with RT libraries and

2 databases.

3   FIG. 9 depicts a reference calibration chromatogram (GC-FID).

4   FIG. 10 depicts a local calibration chromatogram (GC-AED).

5   FIG. 11 shows the comparison of early calibration peaks between the GC-FID

6   and GC-AED systems.

7   FIG. 12 depicts RTL I residual mismatch in RT for a set of calibration peaks

8 from reference and local calibration chromatograms.

9   FIG. 13 depicts the difference between calibration peak RTs on the local (AED)

10 and the reference (FID) GC systems vs run time.

11   FIG. 14 shows an example calibration screen of the RTL II method.

12   FIG. 15 shows a smoothed difference curve and first derivative curves for the

13 RTL II transformation process.

14   FIG. 16 shows the RT match of calibration chromatograms after employing the

15 instrumental correction of RTL I or RTL I plus RTL II transformation.

16   FIG. 17 depicts a chromatogram of the oxygenates mix used to compare the RTL

17 II approach to RTL I only.

18   FIG. 18 depicts a chromatogram of the aromatics mix used to compare the RTL II

19 approach to RTL I only.

20   FIG. 19 depicts a local calibration chromatogram on a 250 µm column.

21   FIG. 20 shows the RT matching of the oxygenates mix on 530 µm and 250 µm

22 columns with same column diameter to film thickness ratio (beta) using the RTL II vs the

23 RTL I method.

24   FIG. 21 shows the calibration chromatograms for the 530 µm reference column

25 (lower trace) and the 250 µm local column with mismatched beta (upper trace).

26   FIG. 22 depicts the reference calibration chromatograms for the 530 µm reference

27 GC column used for calibration of boiling point.

28   FIG. 23 depicts an example calibration screen for the transformation of x-axis to

29 boiling point.

30   FIG. 24 shows the conversion of x-axis unit from time to boiling point with the

31 RTL II method.

32   FIG. 25 depicts an orange oil chromatogram corrected using the RTL II method

33 and calibration with (unknown) peaks present in sample.

1   **FIG. 26** shows an expanded view of reference and corrected local orange oil

2 chromatograms.

3   **FIG. 27** depicts a reference calibration on the GC-AED system for diesel fuel

4 analysis.

5   **FIG. 28** shows the n-dodecane peaks from the reference and local calibrations on

6 the GC-AED system for diesel fuel analysis.

7   **FIG. 29** shows the n-dodecane peaks from the reference and local calibrations on

8 the GC-AED system after the RTL II transformation with linear transformation of the y-

9 axis.

10   **FIG. 30** shows a portion of the diesel fuel chromatograms run on the reference

11 and local GC-AED system, with the local chromatogram being corrected in both the x

12 and y axis.

13   **FIG. 31** shows the RT differences between local GC chromatographic data and a

14 reference table of solvent RTs.

15   **FIG. 32** depicts the results of a polynomial regression through difference data

16 from FIG. 31, which then forms the basis of a local system calibration function.

17   **FIG. 33** shows RT differences between local chromatographic data and the

18 reference table of solvent RTs. The top trace is before correction, the bottom trace is after

19 correction of the table (or local data) using the local system correction function.

20   **FIG. 34** depicts some capabilities that may be included in the data processing unit

21 153.

22 **Detailed Description**

23   FIG. 1a depicts the major steps of the RTL II method 100. A reference

24 chromatographic method including all assay parameters, such as column type, mobile

25 phase type or composition, column head pressure or mobile phase flow rate, and

26 temperature program, is developed and validated to satisfaction on a reference

27 chromatographic system. A collection of reference chromatographic data, RTs,

28 responses (chromatograms and/or databases) is then generated using the reference

29 chromatographic method on the reference system (101). A calibration mix of a plurality

30 of calibration compounds with RTs covering the complete RT range of the reference

31 chromatographic method is run on the reference chromatographic system (103). This

32 process is referred to as the reference calibration and generates reference calibration data.

33 The calibration mix should be chosen with consideration of the following guidelines:

1 (1) The retention times of the calibration compounds should evenly space over the

2 entire retention time range of the reference method.

3 (2) In the case of irregular instrument conditions, some of the calibration peaks

4 should be chosen to closely bracket the times of discontinuous changes in instrumental

5 parameter such as oven temperature program rate or carrier gas flow rate changes.

6 (3) The calibration peaks should be at a concentration such that the calibration peaks

7 have sufficient signal response to be easily integrated but are within the capacity limits of

8 the column. Overloading of calibration peaks can degrade quality of the RT matching.

9 (4) Homologous series (like n-alkanes or methyl esters) make convenient calibration

10 compounds, but any compounds can be used as long as the compounds are

11 chromatographically well behaved.

12 (5) Calibration compounds should be chosen such that the calibration compounds are

13 similar to the polarity of the samples to be analyzed. In the case of a wide range of

14 polarity in a sample mixture, for example alkanes plus alcohols, compounds in the middle

15 of the polarity range, (like methyl esters in this example), should be chosen.

16 (6) The concentration of a chosen calibration peak may be adjusted to be different

17 from the concentration of other calibration peaks so that the chosen calibration peak may

18 be more easily identified by subsequent users. In general, a locking peak used for RTL I

19 is often made the tallest peak in the calibration mix. This makes the locking peak easily

20 recognizable both visually and by automated methods.

21 The calibration mix may also provide reference response factors for standard

22 compounds of interest and for generating response factor correction functions.

23 When a user wants to implement the reference chromatographic method on

24 another chromatographic system (a local system), the user sets up the reference

25 chromatographic method in a local chromatographic system and preferably, but not

26 necessarily, locks the reference chromatographic method using an instrumental correction

27 technique such as the RTL I method (105). The user then runs the reference calibration

28 mix in the local system to create local calibration data (107). This process is referred to

29 as the local calibration. The RTL II software uses a mathematical relationship between

30 the reference and local calibrations to construct local system correction functions which

31 include an x-axis and a y-axis correction function (109). When a unknown sample is run

32 on the local system using the reference chromatographic method, local chromatographic

33 data is generated (111), the local system correction function is then applied to the local

34 chromatographic data to improve matching between the local chromatographic data and

1   the reference chromatographic data (113). The corrected local chromatographic data is

2   then used to generate desired information such as qualitative identification, quantitative

3   analysis, product integrity decisions, and database storage, for example (115). In the RTL

4   II method, the peak areas of the local chromatographic data are preserved in the corrected

5   local chromatographic data, even though the RTs have been adjusted by the RTL II

6   process to match the reference chromatographic data.

7   FIG. 1b depicts a local chromatographic apparatus 150 containing the RTL II

8   algorithms. The local calibration data are produced by the chromatographic hardware

9   151 and combined with the reference calibration data in data processing unit 153 to

10   generate local system correction functions. The local system correction functions are

11   then stored in the data processing unit 153 and are applied to local chromatographic data

12   to produce the corrected local chromatographic data.

13   FIG. 2 depicts the construction of the local system correction functions from the

14   local and the reference calibration data (109). The construction process comprises the

15   following steps:

16   obtaining the reference calibration and the local calibration data (201);

17   shifting the baseline of the local calibration data to zero to prevent exaggerated

18   baseline drift during response correction (203); this step is optional;

19   constructing a smoothed x-axis correction function over the time range of interest

20   using an appropriate mathematical function based on a plurality of data pairs from the

21   reference calibration and the local calibration data (205);

22   constructing a y-axis correction function that is the first derivative of the x-axis

23   correction function (207).

24   For example, a smoothed correction function can be constructed from RT

25   differences in data pairs of the local calibration and reference calibration data as a

26   function of run time, by using a standard curve fitting algorithm. It may be necessary to

27   extrapolate the local system correction function to extend over a local chromatographic

28   time frame of interest. Higher-order polynomial regressions and cubic splines often fit

29   such data well, although other curve fitting approaches are also effective.

30   FIG. 3 depicts the application of the local system correction function to the local

31   chromatographic data in step 113. The application process comprises the following steps:

1   determining a retention time correction (Δrt) from the smoothed x-axis correction

2 function of step 205 based on a local retention time (rt) in the local chromatographic data

3 (301);

4   copying the y value that is at retention time (rt + Δrt) in the local chromatographic

5 data to rt (303);

6   multiplying copied y value by (1+ dΔrt/drt) to preserve original peak area, where

7 dΔrt/drt is determined from a first derivative of the smoothed x-axis correction function

8 (305);

9   repeating steps 301 to 305 with each data point in the new local chromatographic

10 data to create corrected local chromatographic data (307).

11   In another embodiment, additional steps are performed before step 205 to simplify

12 the subsequent RTL II process, when the difference in time scales of the reference

13 calibration and local calibration data is large. These additional steps include:

14   (a) determining a time-axis (retention time) transformation such as a simple linear

15 function, $x' = mx + b$, which makes the RT of the first and last peaks of the local

16 calibration data match those of the reference calibration data exactly;

17   (b) applying the time-axis transformation to the local calibration data to create a

18 time-axis transformed local calibration data; and

19   (c) dividing the y value of each time point in the time-axis transformed local

20 calibration data by m (the slope of the simple correction function) to produce transformed

21 local calibration data with corrected local peak areas.

22   The transformed local calibration data is then used to replace the local calibration

23 data in step 109 to generate a more refined local system correction function.

24   In this embodiment, the local chromatographic data is also subject to the time-axis

25 transformation and y value correction as described above in step (b) and (c), prior to the

26 application of the local system correction function in step 113.

27   The RTL II method may be used concurrently with a method translation technique

28 to correct chromatographic data. Method translation is a technique for converting a

29 method to run on (1) columns of other dimensions but having the same type of stationary

30 phase; (2) columns operated at a different speed of analysis; and (3) GC systems of

31 different detector operating pressures. The most important features of method translation

32 is that the method translation maintains the same relative elution order of analytes and

33 yields predictable retention time changes.

1   Implementation of method translation is described in detail in U.S. Patent. No.

2   5,405,432 to Snyder et al., which is incorporated herein by reference.

3   FIG. 4 is a flowchart showing the utilization of the RTL II method with the

4   method translation technique. First, reference chromatographic data is created on a

5   reference GC system using a reference chromatographic method and a reference column

6   (401). The reference chromatographic method is then translated into a local

7   chromatographic method by performing the method translation process on a local GC

8   system with a local column (403). An instrumental correction method such as RTL I may

9   be performed (405) prior to the RTL II method to further improve the correlation between

10   the local chromatographic data and the translated reference chromatographic data. Local

11   calibration data is subsequently created on the local GC system using the translated (and

12   perhaps instrumentally corrected) local chromatographic method (407). The local and

13   reference calibration data are used to generate local system correction functions (409).

14   The local system correction functions are then applied to locally generated

15   chromatographic data to produce corrected local chromatographic data (411). When

16   using translated conditions, the corrected local chromatographic data are simultaneously

17   adjusted through the RTL II process to match the reference RTs, greatly facilitating

18   subsequent comparison of the local chromatographic data and the reference

19   chromatographic data (413).

20   As shown in FIG. 5, the RTL II method may also include the transformation of the

21   x-axis (RT, migration time, elution volume, etc.) of chromatographic data as required for

22   specific applications, while maintaining the original peak areas. For example, when

23   performing simulated distillation analysis where one replaces RT with boiling point

24   temperature, in capillary gel electrophoresis where one replaces migration time with

25   daltons, and in gel permeation chromatography where one replaces retention time with

26   molecular weight. Reference calibration data and local calibration data are created (501

27   and 503). A unit transformation is applied by replacing the original x-axis unit of either

28   or both of the reference calibration data and the local calibration data with a new x-axis

29   unit (505). Modified local system correction functions are generated (507) which include

30   a function for reassigning the x-axis units in the local data. The modified local system

31   correction functions are then used to generate corrected local chromatographic data with

32   the new x-axis unit. In another embodiment, the x-axis transformation may be performed

33   as a separate step after the standard RTL II correction process.

1   Similarly, the y-axis unit can be transformed by replacing an original unit in the
2   reference calibration data and the local calibration data with a new y-axis unit before the
3   construction of the modified local system correction functions. Examples of the new y-
4   axis unit are concentration, percent, weight, mass, moles, and mole fraction.

5   Better transformation results may be obtained by applying application-specific
6   time transformation functions should the functions be known a priori, instead of empirical
7   functions that are generated based on local calibration data. Examples of such a priori
8   known functions are fundamental principles of chromatography, such as the relationship
9   of flow rate to temperature in GC, or the accepted process of calculating Kovats retention
10   indices using a log function for isothermal conditions and a linear function for
11   temperature programmed conditions. An embodiment that is application-specific is the
12   use of a direct linear relationship to convert the retention time axis to boiling point
13   temperature.

14   The RTL II transformation can also be used to aid in comparison of complex
15   chromatographic data for which no RTL II calibration standards have been run. In an
16   embodiment, peaks found in the chromatographic data itself are used as substitute of the
17   calibration compounds. FIG. 6 depicts an embodiment of a RTL II transformation of
18   chromatograms without the use of reference calibration mixtures. Peaks in a reference
19   chromatographic data are identified by their corresponding RTs (601). Local
20   chromatographic data is then created that contains several recognizable peaks that
21   correspond to those identified in the reference chromatographic data (603). Local system
22   correction functions are created based on the RTs of the corresponding same peaks from
23   the reference and local chromatographic data (605). The local system correction
24   functions are then applied to the same or other local chromatographic data to create
25   corrected local chromatographic data that better correlates to the reference
26   chromatographic data (607).

27   When comparing chromatographic data either visually or mathematically between
28   chromatographic systems, the RTL II transformation described thus far results in precise
29   matching of the x-axis and maintenance of peak areas. However, there is still a need to
30   match the y-axis in some applications as well. For example, in quality control
31   applications it would be desirable to have multiple chromatographic systems produce
32   precisely the same corrected data when the same sample is run on these chromatographic
33   systems . Having the x and y axes matched enables use of consistent response factors for
34   analyte quantification and facilitates visual inspections for peaks that are too large or

1 small or the appearance of new ones. Chromatographic profiles or fingerprints are more

2 consistent across instruments and system configurations. Pattern recognition,

3 chromatographic data subtraction, and ratioing are also made much easier by y-axis

4 correction.

5 FIG. 7 depicts an embodiment that combines y-axis transformation with the RTL

6 II method. The embodiment uses a smoothed transformation of the y-axis of the

7 corrected chromatographic data. In this case, a calibration mix that contains the same

8 calibration compounds at the same concentrations is used to create both the reference

9 calibration data (701) and the local calibration data (703). Local system correction

10 functions are generated by a standard RTL II process as described in FIG. 2 and is applied

11 to the local calibration data to adjust the x-axis and to correct the corresponding peak

12 areas (705 and 707). A y-axis local system response correction function is then

13 determined based on the x-axis and area corrected local calibration data and the reference

14 calibration data (709). The y-axis local system response correction function then

15 becomes part of the RTL II transformation method for local chromatographic data (711-

16 717). Applying both the local system correction functions (x-axis and y-axis) and the

17 local system response correction function (y-axis) to local data makes the corrected local

18 data matches that of the reference calibration data better.

19 The process described above will correct for the differences in peak responses

20 between the local and reference systems. An optional additional step is to offset the

21 entire corrected chromatographic data to have the same baseline level as the reference

22 system.

23 In an embodiment, RTL II method is written as an automated software program

24 such as a ChemStation macro. The local calibration data and local system correction

25 functions are generated and used on the local system. Whenever local chromatographic

26 data is to be corrected, the macro uses the local system correction functions to perform

27 the steps such as described in FIGS. 1-7 on the local chromatographic data in question to

28 match the RTs (and response factors) to the reference system. The ChemStation macro

29 may be stored in the data processing unit 153. FIG. 34 depicts some capabilities that may

30 be included in the data processing unit 153.

31 The principle behind the RTL II method is to recalculate chromatographic data to

32 make the chromatographic data match a reference. For applications like those described

33 in U.S. Patent No. 5,827,946 to Klee et al., the goal is to make the RTs from a specific

34 chromatographic setup match precisely those in a library or database. The more closely

1   the RTs match, the smaller the time window that is used to search the database for the

2   identity of an unknown. With smaller time windows, the more likely the search will yield

3   a single result for unknown identification and the more accurate the identification is

4   expected to be. Whenever time windows are wide enough to produce multiple possible

5   identities for an unknown, other analyses are required to finish the identification.

6   The RTL II method could be used to advantage in this application. The reference

7   calibration data may be the retention times of compounds selected from a database.

8   These same compounds may then be used for the local calibration and the RTL II process.

9   It is also possible to do the reverse. Instead of recalculating the chromatographic

10   data to match the database, the RTs in the database can be recalculated to match the local

11   system. Sometimes, RT databases have fewer entries than a local analysis has peaks. In

12   these cases, the recalculation is much faster because each local peak is comprised of

13   multiple data points that would otherwise be corrected. However, conversion of the

14   database would need to be performed only once, after which it could be repeatedly used

15   for all library searches done locally.

16   FIG. 8 depicts a method to reduce or eliminate the difference in RTs between a

17   local system and a reference database or library. The method comprises the following

18   steps to create corrected local chromatographic data or corrected reference database or

19   library:

20   providing a RT database (801);

21   optionally locking the local system to the RT database using an instrumental

22   method such as the method described in U.S. Patent No. 5,827,946 to Klee et al (803);

23   running a calibration mix on the local system (805);

24   constructing local system correction functions to minimize differences between

25   the local calibration data and the reference database values for the calibration mix (807);

26   generating local chromatographic data (809);

27   applying the local system correction functions to adjust the local chromatographic

28   data (811);

29   using the corrected local chromatographic data to search the reference database to

30   obtain more unique or accurate search results for each sample (813).

31   Steps 811 and 813 may be repeated to correct and search each local

32   chromatogram. Alternatively, the local system correction functions may be applied to the

33   reference database by the following steps:

1   applying the local system correction functions to adjust the reference database

2   values to generate a local reference database whose values closely match those of the

3   local system (815);

4   using the local chromatographic data directly to search the local reference

5   database (817).

6   *Example 1, Creation of a reference calibration*

7   A calibration mix was run on a reference GC with a flame ionization detector

8   (GC-FID system) to create a reference calibration. The calibration mix contains four

9   oxygenates and 13 n-alkanes in approximately equal volumes with the exception of n-

10   dodecane, which will be used as the locking peak for RTL. The column characteristics,

11   assay method, and reference chromatogram are shown in FIG. 9.

12   *Example 2, Creation of a local RTL II calibration*

13   In this example, the assay method used in Example 1 was duplicated on a local

14   GC system with an atomic emission detector (GC-AED system). The calibration mix

15   was run on the GC-AED system and the dodecane peak was locked using RTL I to be

16   20.850 min, matching that on the reference GC-FID system. FIG. 10 shows the local

17   calibration data on the GC-AED system.

18   *Example 3, Residual RT differences With RTL*

19   This example shows the comparison of early calibration peaks from the reference

20   GC-FID system and the local GC-AED system. FIG. 11 illustrates that, even though RTL

21   locks the n-dodecane peak in both local (GC-AED) the and reference (GC-FID)

22   chromatographic data near 20.850 minutes, there are still residual RT mismatches,

23   especially at the extreme ends of the RT range of 2 to 7 min in this example.

24.   FIG. 12 shows the residual mismatch of the n-hexane peak in more detail near

25   6.35 min. The difference in measured retention time is 0.017 minutes. This difference is

26   large enough to result in the misidentification of peaks that elute close together. The

27   difference in RT seen in FIG. 12 is what the RTL II method is intended to reduce or

28   eliminate.

29   FIG. 13 shows a graph of the measured difference in RT for all the calibration

30   peaks between the local (AED) and reference (FID) systems. The differences range from

31   + 0.029 minutes to -0.027 minutes.

32   The shape of the difference curve in FIG. 13 indicates that the largest proportion

33   of the errors is not random in nature, but is the result of some systematic bias. It should

1   therefore be possible to remove a substantial portion of the RT differences between the

2   two systems even for components not in the calibration mix.

3   *Example 4, RTL II transformation of the local calibration data*

4   FIG. 14 shows an example calibration screen from a typical data acquisition and

5   reporting software. The correction function after linear transformation - extrapolation of

6   the ends and curve fitting (e.g., splining in this example) - is shown in FIG. 15. Also

7   shown in FIG. 15 are the first derivative curves, both before and after splining.

8   FIG. 16 shows the same two chromatograms from FIG. 12 in Example 3 with the

9   corrected version of the local AED calibration added. Note that the retention time now

10   matches much more closely. The agreement of RTs for the calibration mix after RTL I

11   and RTL II is further demonstrated in Table 1.

12

13   <u>Table 1. Agreement of RTs with the RTL I and RTL II method for the calibration mixture</u>

	FID RT	AED RTL I	AED RTL II	RTL I Difference	RTI II Difference
methanol	2.138	2.168	2.138	0.030	0.000
ethanol	2.685	2.707	2.685	0.022	0.000
acetone	3.081	3.097	3.081	0.017	0.001
n-pentane	3.570	3.578	3.570	0.008	0.000
MTBE	5.166	5.150	5.166	-0.016	0.000
n-hexane	6.359	6.334	6.359	-0.025	0.000
n-heptane	9.652	9.625	9.652	-0.027	0.000
n-octane	12.522	12.500	12.522	-0.022	0.000
n-nonane	14.972	14.955	14.972	-0.017	0.000
n-decane	17.123	17.111	17.123	-0.012	0.000
n-undecane	19.063	19.054	19.063	-0.009	0.000
n-dodecane	20.844	20.839	20.844	-0.005	0.000
n-tridecane	22.494	22.494	22.494	0.000	0.000
n-tetradecane	24.037	24.042	24.037	0.005	0.000
n-pentadecane	25.488	25.496	25.488	0.008	0.000
n-hexadecane	26.854	26.871	26.854	0.017	0.000
n-heptadecane	28.239	28.262	28.239	0.023	0.000

14   _____

15

16   *Example 5, Testing the RTL II versus RTL I performance*

17   This example demonstrates the matching effect of RTL II verses RTL I. A test

18   mix containing several oxygenated compounds was run on the GC systems described in

1   Example I using the same assay method. FIG. 17 shows the chromatogram of the test

2   mix run on the local GC system.

3   The quality of RT matching for the oxygenates mix on the AED local system to

4   the reference FID system with both the RTL I and RTL II processing is compared in

5   Table 2.   The RTL II method makes a substantial (tenfold) improvement in RT matching

6   over the RTL I method.   The average of the absolute values of the differences between

7   the two systems using the RTL I method is 0.021 minutes.   Creating corrected

8   chromatographic data using the RTL II method lowers this average tenfold to 0.002 min.

9

10   Table 2. Agreement of RTs with the RTL I and RTL II method for oxygenate mixture

	AED RTL I	530 FID (Ref)	AED RTL II	RTL I Difference	RTL II Difference
methanol	2.167	2.138	2.136	0.029	-0.002
ethanol	2.708	2.686	2.685	0.022	-0.001
acetone	3.097	3.081	3.080	0.016	-0.001
isopropanol	3.263	3.250	3.250	0.013	0.000
methylene chloride	3.917	3.913	3.916	0.004	0.003
methyl tert-butyl ether	5.150	5.165	5.166	-0.015	0.001
iso-propyl ether	6.294	6.320	6.319	-0.026	-0.001
ethyl acetate	6.392	6.416	6.418	-0.024	0.002
1,2-dichloroethane	7.244	7.271	7.272	-0.027	0.001
ethylene glycol dimethyl ether	7.626	7.653	7.655	-0.027	0.002
iso-octane	9.323	9.354	9.351	-0.031	-0.003
pyridine	10.242	10.268	10.269	-0.026	0.001
1,3-butanediol	13.267	13.288	13.288	-0.021	0.000
nitrobenzene	18.537	18.554	18.547	-0.017	-0.007
1,2,4-trichlorobenzene	20.592	20.606	20.597	-0.014	-0.009
Average (absolute values)				*0.021*	*0.002*

11

12

13   FIG. 18 shows the chromatogram of another test mixture containing mostly

14   aromatic compounds run on the local GC-AED system of using the same assay method.

15   The results of RT matching for the aromatics mix on the AED local system to the

16   reference FID system with both the RTL I and RTL II method are summarized in Table 3.

17   The RTL II method again makes a substantial (tenfold) improvement in RT matching

18   over the RTL I method.   The average of the absolute values of the differences between

19   the two systems using the RTL I method is 0.018 minutes.   Creating corrected

20   chromatographic data using the RTL II method lowers this average tenfold to 0.0013

21   min.

22

1   <u>Table 3. Agreement of RTs with the RTL I and RTL II method for aromatics mixture</u>

	AED RTL I	530 FID (Ref)	AED RTL II	RTL I Difference	RTL II Difference
n-hexane	6.339	6.367	6.365	-0.028	-0.002
	7.274	7.303	7.302	-0.029	-0.001
benzene	8.062	8.091	8.091	-0.029	0.000
cyclohexane	8.385	8.416	8.414	-0.031	-0.002
	9.216	9.245	9.244	-0.029	-0.001
n-heptane	9.622	9.651	9.649	-0.029	-0.002
toluene	11.346	11.371	11.371	-0.025	0.000
n-octane	12.497	12.520	12.519	-0.023	-0.001
ethylbenzene	13.889	13.910	13.908	-0.021	-0.002
m and p-xylene	14.103	14.122	14.122	-0.019	0.000
styrene	14.539	14.557	14.557	-0.018	0.000
o-xylene	14.674	14.693	14.692	-0.019	-0.001
	14.953	14.969	14.970	-0.016	0.001
iso-propylbenzene	15.413	15.430	15.429	-0.017	-0.001
propylbenzene	16.082	16.098	16.096	-0.016	-0.002
	16.227	16.242	16.241	-0.015	-0.001
a-methylstyrene	16.598	16.612	16.611	-0.014	-0.001
tert-butylbenzene	16.951	16.966	16.963	-0.015	-0.003
n-decane	17.110	17.121	17.122	-0.011	0.001
sec-butylbenzene	17.348	17.361	17.360	-0.013	-0.001
	18.048	18.058	18.059	-0.010	0.001
butylbenzene	18.220	18.231	18.231	-0.011	0.000
n-undecane	19.053	19.060	19.062	-0.007	0.002
n-dodecane	20.838	20.839	20.842	-0.001	0.003
n-tridecane	22.494	22.491	22.495	0.003	0.004
		Average (absolute values)		***0.018***	***0.0013***

2

3

4   *Example 6, Using the RTL II method with translated methods: Same Beta*

5   This example demonstrates the use of the RTL II method with the translated

6 method. In this example, the reference system is again the same 530 μm DB-1 system as

7 used in earlier examples. The local system is a GC-FID system with a DB-1 column that

8 is the same length (30 m) but is 250 μm in diameter and with a film thickness of 1.42 μm.

9 Using the method translation technique, a set of conditions were calculated to give the

10 same nominal retention times on the 250 μm column as on the 530 μm column. The 250

11 μm column was then locked with n-dodecane to elute at 20.850 min. Note that to achieve

12 the same retention times through simple instrumental locking processes link RTL I, it is

13 necessary that ratio of the column diameter to the film thickness is the same (i.e. the same

14 beta) for the two columns, as is the case here.

15   FIG. 19 shows the local calibration chromatogram on the 250 μm column. The

16 reference calibration chromatogram is shown in FIG. 9. The oxygenates test mixture was

17 then used to evaluate the quality of RT matching with both the RTL I and RTL II method.

1   FIG. 20 and Table 4 below shows the improvement in RT matching provided by the RTL

2   II method. As seen in Table 4, the improvement in RT matching with the RTL II method

3   is significant. The differences in RTs are reduced on average fivefold.

4

5   <u>Table 4. Agreement of oxygenate mix RTs with the RTL I and RTL II method for</u>

6   <u>columns of different diameter but same beta</u>

	250 RTL I	530 FID	250 RTL II	RTL I Errors	RTL II Errors
methanol	2.133	2.138	2.132	-0.005	-0.006
ethanol	2.656	2.686	2.684	-0.030	-0.002
acetone	3.034	3.081	3.080	-0.047	-0.001
isopropanol	3.196	3.250	3.247	-0.054	-0.001
methylene chloride	3.829	3.913	3.901	-0.084	-0.012
methyl tert-butyl ether	5.047	5.165	5.167	-0.118	0.002
iso-propyl ether	6.194	6.320	6.315	-0.126	-0.005
ethyl acetate	6.281	6.416	6.402	-0.135	-0.014
1,2-dichloroethane	7.128	7.271	7.243	-0.143	-0.028
ethylene glycol dimethyl ether	7.526	7.653	7.638	-0.127	-0.015
iso-octane	9.256	9.354	9.356	-0.098	0.002
pyridine	10.149	10.268	10.240	-0.119	-0.028
1,3-butanediol	13.200	13.288	13.255	-0.088	-0.033
nitrobenzene	18.494	18.554	18.503	-0.060	-0.051
1,2,4-trichlorobenzene	20.563	20.606	20.557	-0.043	-0.049
Average (absolute values)				*0.085*	*0.017*

7

8

9   ***Example 7, Using the RTL II method with Translated Methods: Mismatched Beta***

10   A more difficult application of the RTL II method is the situation where the

11   method is translated to a column with a different beta. In this example, the experiment

12   described in Example 6 was repeated using a 250 μm DB-1 column as above except with

13   a film thickness of 1.00 μm instead of 1.42 μm. This 40% mismatch in beta means that

14   the RTL I method cannot be used effectively, because adjusting the inlet pressure to make

15   the n-dodecane locking peaks match would change the flow so much as to potentially

16   change the elution order of some peaks. Therefore, this is an example of where the RTL

17   II method can be used to unique advantage.

18   The reference is again the 530 μm column whose calibration is shown in FIG. 19

19   FIG. 21 shows the two calibration chromatograms. The 250 μm (local) calibration is the

20   upper trace and the 530 μm reference (from FIG. 9) is the lower. Use of the RTL II

21   method reduced the time difference for the oxygenates mixture to an average of 0.011

22   minutes and for the aromatics to an average of 0.010 minutes. This is a greater than 100

23   fold reduction in the RT differences compared to that shown in FIG. 21.

1   Please note that the differences in RTs from the local to the reference are so large

2   in this example that the peak areas change substantially in the corrected chromatographic

3   data if the response corrections discussed earlier (and in FIGS. 3 and 7) are not used. The

4   change in area was as large as 20% without using the first derivative correction.

5   Incorporating the first derivative correction dropped the measured change in area to

6   below the experimental error in integrating the peaks (~1%).

7   *Example 8, Transformation of X-Axis to Other Units*

8   This example shows the transformation of x-axis units by the RTL II method.

9   FIG. 22 is a chromatogram of a mixture of n-C5 through n-C16 alkanes run on GC-AED.

10   The peaks are labeled with RTs determined by a ChemStation integrator. To convert

11   chromatographic data run on this system to a boiling point x-axis, the retention times in

12   the reference calibration are replaced with the boiling points (in this example °C) of each

13   alkane.

14   An example RTL II calibration screen for converting to boiling point on the x-axis

15   is shown in FIG. 23. The reference retention times had previously been replaced in this

16   screen by the boiling points.

17   FIG. 24 shows the corrected chromatographic data of the same alkanes before and

18   after transformation to boiling point. One handy feature of the process is that the

19   ChemStation integrator now labels peaks with temperature. This type of display should

20   be useful to petroleum refiners because they can easily read the boiling point of a

21   compound directly from the corrected chromatographic results.

22   Other conversions that may be performed with this approach include retention

23   index and molecular weight. Note that in some retention index calculations, log

24   interpolation is used during isothermal sections of the chromatogram and linear

25   interpolation during the temperature programming. This approach can be used in the

26   calculation of corrected chromatographic data as an option, but corrections in the

27   transition zones from one algorithm to the other become less accurate because they are

28   more difficult to curve fit.

29   *Example 9, Transformation of chromatographic data without calibration data*

30   The RTL II calculation can be used to aid in comparison of complex

31   chromatographic data for which no calibrations have been run. This example shows the

32   comparison of two chromatograms of orange oil run on two different columns using the

33   RTL II method. The columns are of different diameters, 320 and 530 μm. The 530 μm

1   GC conditions were calculated using the method translation to yield runs that were two

2   times faster than with the 320 μm column.

3   The reference calibration was created by entering the retention times of several

4   recognizable (although unknown) peaks in the 320 μm chromatogram and then entering

5   the corresponding RTs of the same peaks from the 530 column for the local calibration.

6   FIG. 25 shows the two original chromatograms and the corrected 530

7   chromatogram for comparison. FIG. 26 shows an expanded view of the 320

8   chromatogram and the corrected 530 chromatogram. The lower resolution of the 530

9   corrected chromatogram reflected the reduced chromatographic resolution of the larger

10   diameter column and conditions and is not an artifact of the RTL II method.

11   This example shows the utility of the RTL II method for improving graphical

12   comparison of chromatograms.

13   *Example 10, Incorporating y-axis transformation of chromatographic data into the*

14   *RTL II method*

15   In this example, two identical GC-AED systems were set up to analyze diesel

16   fuels. The columns were run in constant flow mode. The RT differences between the

17   reference and local calibrations ranged from 0.003 – 0.300 minutes, and the RTL I was

18   not used. The reference calibration chromatogram is shown in FIG. 27.

19   FIG. 28 shows an expanded view of the n-dodecane peaks from the reference and

20   local calibrations. The difference in gain and offset in the peak response (y-axis) and RT

21   (x-axis) are clearly visible.

22   FIG. 29 shows the same peak from FIG. 28, but after the local chromatographic

23   data has been x-axis corrected and then y-axis corrected. The two peaks now

24   superimpose nicely.

25   FIG. 30 shows a portion of diesel fuel chromatograms run on the two systems.

26   The shown chromatogram from the local AED system was corrected in both the x and y

27   axes using RTL II. Again, the two chromatograms now superimpose very well.

28   *Example 11, Variant of invention for application to RT libraries and databases*

29   This example demonstrates the reduction or elimination of the difference in RTs

30   between a local GC system and a database or library (reference system). FIG. 31 shows a

31   plot of the difference between the measured (local) retention times as a function of run

32   time on a local GC and those in a database of solvent retention times. The deviations

1   from the table range from about –0.02 min to 0.12 min.   This means the time window

2   should be at least 0.15 minutes for searching unknowns against the database.

3   FIG. 32 shows the third through eighth order polynomial regression results for the

4   RT difference data. The fifth through the eighth orders all fit the difference function well.

5   The eighth order was chosen to correct the RT table.

6   FIG. 33 compares the time differences between the local system and both

7   corrected table and the original table. With the corrected table, the search time window

8   could be reduced to about 0.05 minutes, representing nearly a threefold improvement.

9   Although a number of embodiments and their advantages have been described in

10   detail, various changes, substitutions and alterations can be made herein without

11   departing from the scope of the RTL II process as defined by the appended claims and

12   their equivalents.

13